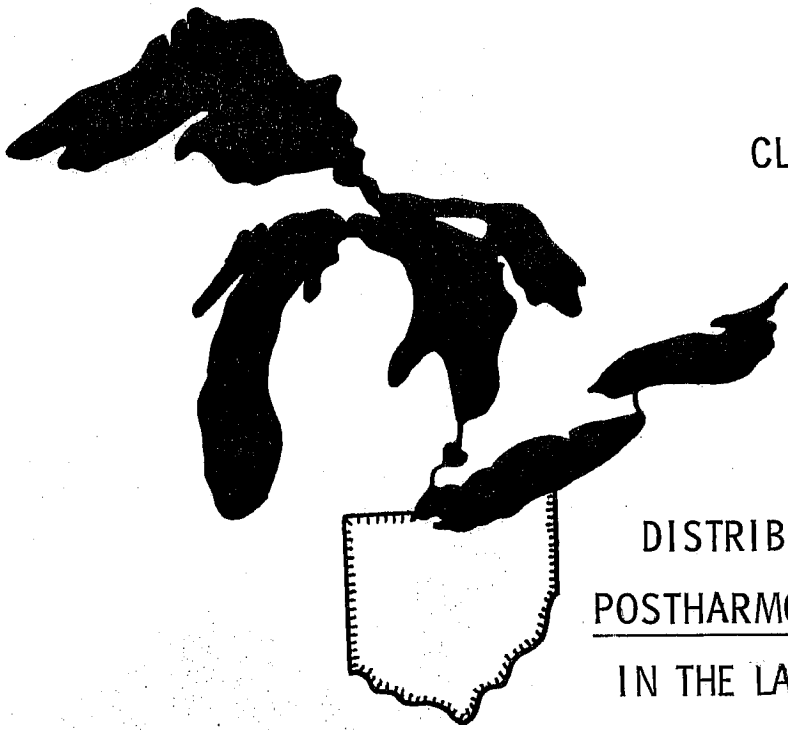


CLEAR TECHNICAL REPORT NO. 73



DISTRIBUTION OF METACERCARIAE OF
POSTHARMOSTOMUM HELICIS (TREMATODA)
IN THE LAND SNAIL ANGUISPIRA KOCHI
STRONTIANA FROM GREEN ISLAND,
LAKE ERIE

by

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PREFACE

The following document was prepared by Benjamin N. Tuggle as partial requirement for a Master of Science Degree in the Department of Zoology, The Ohio State University. Research conducted for this thesis was partially supported by the Center for Lake Erie Area Research and the Franz Theodore Stone Laboratory. Dr. John L. Crites served as advisor; Drs. N. Wilson Britt, Walter E. Carey, and Charles E. Herdendorf served as members of the reading and examination committee.

On behalf of the Center for Lake Erie Area Research, I am pleased that we are able to reproduce copies of this research report and make them available to other scientists.

Charles E. Herdendorf
Director

ACKNOWLEDGEMENTS

I am grateful to all the people who helped me directly and indirectly in the completion of this thesis. I am particularly grateful to Dr. John L. Crites whose patience, guidance, advice and concern was instrumental in the completion of this study.

To Stan Eisen, Rey Farve, Ted Spellmire, and John Wehrmeister, I give my thanks for their help in collecting snails, trapping mice, and accompanying me to Green Island.

I also give thanks to Dr. Charles E. Herdendorf, Dr. Walter E. Carey, and Dr. Wilson Britt who read this thesis and contributed helpful ideas.

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INTRODUCTION

Green Island is a 7 hectare island which is adjacent to the Bass islands in Lake Erie. The island has been uninhabited since 1923 and now belongs to the Ohio Department of Natural Resources which maintains it as a reserve. There were numerous oral reports of large numbers of shell-bearing land snails distributed all over the island floor.

This island was chosen because it has been uninhabited for over 50 years, and the snail population had never been examined for parasites. The author hypothesized that Postharmostomum species, a trematode which utilizes terrestrial snails as intermediate hosts and mice as definitive hosts, might be present in the snails since this trematode was reported from the Erie islands by Miller (1935).

The objectives of this study were to:

- 1) record the snail species present on Green Island.
- 2) determine the geographic distributions and density of snails in particular areas.
- 3) identify the parasitic stages found in the snail population.
- 4) compare the incidence and intensity of the parasites in snail hosts in particular zones on the island.
- 5) examine the intensity and frequency of infection in different sizes of snails which harbor parasites.
- 6) examine the possibility of statistical correlations of height, diameter and weight of the snails with degree of infection.

7) develop a multiple factor regression equation to estimate the mean number of parasites in the snails collected from specific zones with a specific height, diameter and weight.

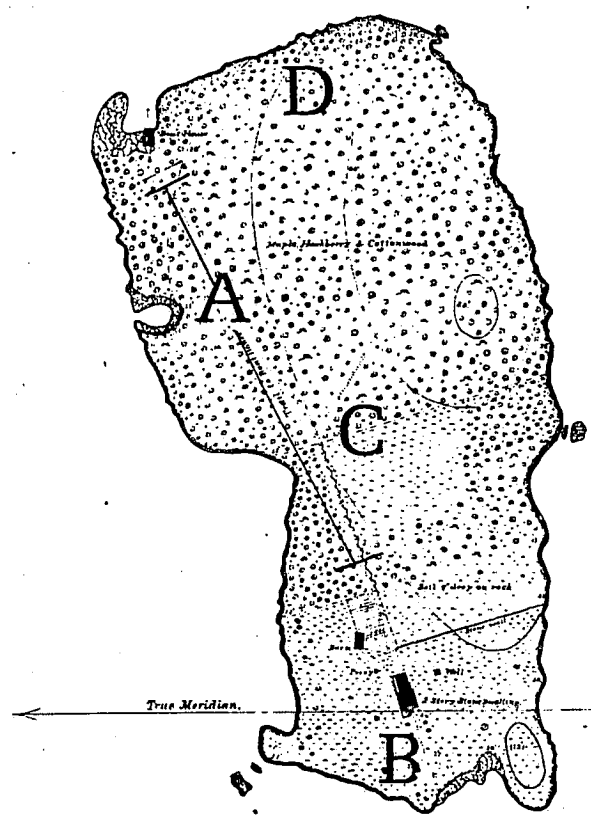
METHODS AND MATERIALS

Five hundred and fifty, terrestrial, shell-bearing snails were collected from June through August in 1976, from Green Island, Ottawa County, Ohio in Lake Erie. The snails collected were keyed to Anquispira kochi strontiana (Clapp 1916) using Taft (1961). All of the snails found alive were of this genus except one Haplotrema concavum that was found in front of the entrance of a cave on the island. An extensive search to find other live shell-bearing lands snails revealed only the above species. Although large numbers of A. K. strontiana were found on various parts of the island, they were only collected from the areas designated below. These areas were chosen because of their geographical and vegetative differences.

An area designated A consisted of a zone which extended approximately 35 meters inland from the eastern shore of the island and ended approximately 55 meters away from an 113 year old, abandoned lighthouse (Text Figure 1). Snails were collected from a range of approximately 10 meters on either side of a concrete walkway which runs from the eastern shore to the western shore of the island, throughout the zone which comprised area A.

An area designated B consisted of an area approximately 15 to 20 square meters which was located on the western side of the lighthouse on the western shore of the island, formerly a grass lawn.

LAKE ERIE

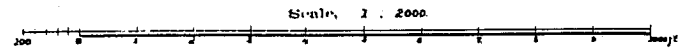


NOTE.

Latitude of Station.....	41 38' 44"
Longitude of Station.....	82 32' 04"
Kind of Light.....	Const.
When built.....	1854.
When rebuilt or renovated.....	1864.
Order of Light.....	4th
Characteristic of Light.....	FWvar with WF.
Base of Tower above water level.....	14 ft.
Focal Plane above water level.....	57 ft.
Area of Reservation.....	171 Acres.
Area enclosed.....	39 "
Island purchased.....	1833.
Fences.....	-----
Walks, Plank.....	-----

Text Figure 1. Map of Green Island Showing Collection Zones.

GREEN ISLAND



An area labeled C was located in the middle of the island some 30 meters from area A. Its collection area was approximately 5 to 10 square meters. Area D was located on the eastern shore of the island. Its collection area was also approximately 5 to 10 square meters.

One hundred snails were examined from area A and 100 from area B. From area C, 150 snails were examined and from area D 65 snails were examined for a total of 415 snails from which data was recorded. The number of snails examined in each zone varied because these numbers of snails were collected from the above zones. Additional trips to Green Island to collect more snails from each zone became difficult because of weather conditions.

The snails were separated according to the area from which they were collected. They were maintained in the laboratory in large aquaria containing moist soil and were fed lettuce and cabbage leaves until they could be examined. Each snail was measured in height and diameter to the nearest millimeter and weighed to the nearest one-hundredth of a gram on a digital precision balance.

The shells of the snails were crushed and removed after which the soft parts were placed in Ringer's "cold" solution and examined using a dissection microscope. Dissection of the pericardial region revealed that the snails were harboring a metacercarial stage of a distomate trematode. The metacercariae were found only in the pericardial region of the snails. The number of metacercariae found in each snail was recorded and later fixed in cold AFA (alcohol, formalin and acetic acid) solution and preserved in 70% ethanol. The trematodes were later stained with Semichon's carmine and mounted in piccolyte. Examinations

of other snail organs failed to reveal any other trematode stage or parasitic helminth infection. Live and stained specimens were examined using a phase contrast microscope. The flukes were tentatively identified as Postharmostomum helicis (Leidy 1847) Robinson 1949.

Twenty slugs were collected from Green Island and identified as Limax maximum. The slugs and the lone Haplotrema concavum found were also examined for parasites but none were found.

In order to positively identify the metacercaria as Postharmostomum helicis efforts were made to trap mice on Green Island, because mice harbored the adult trematode. However, the efforts proved unsuccessful. Therefore, attempts were made to trap mice on South Bass Island so that the metacercaria could be experimentally introduced. One mouse, identified as Peromyscus leucopus, was trapped using a Sherman live trap in a wooded area on South Bass Island. The feces of the mouse were examined for four days using smear techniques to determine if the mouse was infected with P. helicis prior to the experimental infection.

The mouse was fed 5 crushed A. k. strontiana every 2 days for three weeks after which it was autopsied. The entire digestive system was placed in Ringer's "warm" solution and examined.

Ten Peromyscus leucopus were trapped using Sherman live traps on a wooded lot in Columbus, Ohio and housed in metal shoe box cages. Fecal smear examinations were performed, but no parasite eggs could be found. The mice were separated into three groups for experimental infections. Group I contained 4 P. leucopus fed 10 P. helicis metacercaria using a stomach tube. Group II were starved for two days, then

fed ten crushed A. k. strontiana collected from Green Island. This group also contained 4 mice. Group III, the control group, contained 2 P. leucopus. After five weeks the entire digestive tracts of all mice were examined for adult P. helicis.

Experimental infections using Mus musculus were also conducted. The same methods for separation and infection were used as above. All adult trematodes were fixed in cold AFA and placed in 70% ethanol. They were later stained with Semichon's carmine and mounted in piccolyte.

An IBM 370/165 computer was used for analysis of data. Programs were conducted in the Instructional Research Computer Center at The Ohio State University.

Drawings were made with the aid of a Wild drawing tube. Photographs shown in the text were taken with a Minolta 101 camera.

Representative specimens of Postharmostomum helicis, adult and metacercaria, were deposited in the National Parasite Collection in Beltsville, Maryland (USNM Helm. Coll. No. 74489 and 74490).

HISTORICAL BACKGROUND

In 1843, Dujardin established the genus Brachylaima when describing a trematode he found in the intestine of a shrew. Dujardin described a similar trematode in 1845 (cited in Allison 1943) and named it Brachylaima migrans but he later reduced it to the subgenus Brachylaimus under the genus Distomum. Blanchard in 1847 (cited in Allison 1943) changed the spelling of Dujardin's subgenus to Brachylaemus and restored it to generic ranking.

Authors apparently unaware of the genus Brachylaemus grouped all related forms together under the old generic name Distomum. In 1899, Braun created the genus Harmostomum using species that were similar to the flukes once grouped under Dujardin's genus Brachylaemus. He removed them from the old genus Distomum and grouped its related forms under his new genus Harmostomum.

Odhner (1912) established the family Harmostomidae with two subfamilies, Harmostominae and Liolopinae. Odhner used Braun's genus Harmostomum as the type genus for his new family.

The genus Postharmostomum was created by Witenberg in 1923 (cited in McIntosh 1934). Two years later he subdivided the genus Harmostomum into two subgenera, Harmostomum and Postharmostomum. Witenberg (1925) did make reference to the fact that Postharmostomum would be considered as a separate genus because of its distinct morphological differences with Harmostomum.

Authors since then (McIntosh 1934, Miller 1935, Allicata 1940, Robinson 1949, Ulmer 1951) have considered Postharmostomum as a distinct genus because of its winding convolutions of the intestinal ceca which is an important characteristic in identification.

Joyeux and Foley (cited in Robinson 1949b) pointed out that Harmostomum Braun, 1899 was not actually a valid genus rather it was a synonym of Brachylaima Dujardin, 1843 (changed to Brachylaemus by Blanchard, 1847). They stated that Brachylaemus was the valid genus name, and changed the family name to Brachylaemidae.

Allison (1943) established the subfamily Brachylaeminae which had been Harmostominae in the family Harmostomidae established by Odhner (1912).

The earliest reference to the species Postharmostomum helicis was by Leidy (1847) when he described the metacercarial stage of a trematode found in the pericardial cavity of Helix alternata (= Anguispira alternata), and named the fluke Distoma helicis.

An early reference (Witenberg 1925) stated that Meckel in 1846 supposedly described a metacercarial state that he removed from the kidney region of the snail Helix pomatia, however he failed to name it. Whether the trematodes described by Leidy one year later was the same as Meckel's is not known.

Diesing (1850) in an effort to name the fluke described by Meckel, proposed the name Distoma helicis pomatiae. Diesing (1855) (cited in Ulmer 1951) later changed it to Cercariaeum helicis alternatae.

Creplin (1849) (cited in Robinson 1949b) renamed the trematode described by Leidy Distomum pericardium apparently for no reason. Dollfus (1935) stated that D. helicis should remain valid although Creplin changed it to D. pericardium, since D. helicis had not been used before 1847 in any publication. Leidy (1850) described what he thought to be a new species and named it Distomum vagans. Robinson (1949b) pointed out, however that Leidy used two different species of metacercaria in his description of D. vagans and one was merely a developmental stage of the fluke he had previously named.

McIntosh (1934) discovered an adult trematode in the cecum of the chipmunk, Tamias striatus lysteri. He indicates that his species shows some similar characteristics to Leidy's D. vagans, but he also states that Leidy used more than one species when describing his D. vagans. For this reason he proposed the name Postharmostomum laruei for his new species.

Miller (1935) discovered a distomate metacercaria in land snails on South Bass island, Ohio, and after feeding the metacercaria to deer mice got an adult trematode he called Brachylaima (Postharmostomum) sexconvolutum. Ulmer (1951) feels Miller dealt with more than one species of metacercaria when Miller did his study on the life cycle and the growth rate of his new species. Miller (1939) apparently recognizing that his B.(P.) sexconvolutum was a synonym of McIntosh's P. laruei, reported on the growth rate of that trematode under the name P. laruei McIntosh.

Ulmer (1949) and Robinson (1949a) published preliminary descriptions of P. helicis. Ulmer used the name P. laruei for the trematode

he described. Robinson did not use a specific name but did indicate that the trematode could be identical with the metacercaria described by Leidy in 1847 being D. helicis or D. vagans.

Robinson (1949b) when presenting the life cycle of P. helicis agrees that helicis should be the valid name for the metacercaria since it was first described by Leidy in 1847. Despite some references stating that Meckel described it first Robinson agrees that because Leidy named the fluke first, his name should stand. He also separated the two species Leidy designated as D. vagans and showed the relationship of the two developmental stages of D. vagans to D. helicis.

Robinson (1949b) reported that because McIntosh had no reference to D. helicis he was not aware that the larval stage for the fluke he named P. laruei had already been named previously. Therefore he lists D. pericardium, Cercariaeum Helicis alternata, and P. laruei as synonyms of P. helicis.

Ulmer (1951) discovered a mother sporocyst stage in the life cycle of P. helicis that Robinson did not report. He also has contributed most of the information that is known about P. helicis at the present time.

In view of various literature reviews, it is clear that the valid name for the trematode mentioned in this thesis is Postharmostomum helicis (Leidy 1847) Robinson 1949.

LIFE CYCLE OF POSTHARMOSTOMUM HELICIS

Ulmer (1949) and Robinson (1949a) published simultaneous papers with description of a trematode which is now known as Postharmostomum helicis. Neither author knew of the other's discovery or experimental work. Ulmer called his trematode P. laruei but Robinson gave no name for his species. He did however suggest that it could be the metacercaria first described by Leidy (1847).

Robinson discovered the metacercarial stage in the percardial regions of Polygyra thyroidus and Anguispira alternata. Ulmer discovered the metacercaria in the above snails plus P. multilineata, P. profunda, Gastrodonta ligera, and Deroceras laeve. Ulmer and Robinson indicated that the sporocyst stage of the life cycle could only be found in Anguispira alternata. Robinson recovered the adults in the cecum of Peromyscus leucopus while Ulmer used P. maniculatus.

Robinson (1949b) published aspects of the life cycle of P. helicis. He described the egg, miracidia, sporocyst, cercaria, metacercaria, and the adult. He was not aware however of the mother sporocyst later discovered by Ulmer (1951). The following is summarized from Ulmer's (1951) account of the life cycle of Postharmostomum helicis.

The adult worms are found in the cecum of the white-footed, Peromyscus sp. mouse and the eastern chipmunk, Tamias striatus. These are the natural definitive hosts of P. helicis. Sexually mature worms

lay embryonated eggs which are voided in the feces of the definitive host. The eggs are eaten by pulmonate land snails but hatching does not occur unless the eggs are eaten by the land snail Anguispira alternata.

The miracidium which emerges from the egg possesses three groups of cilia located in three distinct regions of the body. The miracidium migrates across the intestinal wall into the hepatic gland and surrounding connective tissue. The miracidia develops into multi-branched mother sporocysts which after seven to eight weeks produce embryos of daughter sporocysts released through birth pores of branches of mother sporocyst. Mother sporocyst disappear in about 10 weeks after infection.

Daughter sporocysts develop in the hepatic gland and kidney of the snail and the branches protrude into the mantle cavity. Daughter sporocysts may remain as long as one year releasing shorttail cercaria year round. The cercaria are released about 12 to 33 weeks after infection depending on the temperature (Elwell and Ulmer 1968).

The cercaria escape the first intermediate host through the respiratory pore and appear in the slime trail of the snail. The cercaria must enter a snail which is not infected by a sporocyst in order to develop because they will not develop in a snail which harbors one. Snails which cross the slime trails of infected first intermediate hosts are infected when the cercaria enter the pore of the primary ureter. The cercaria migrate up the ureters into the kidney and into the pericardial region via the reno-pericardial connection.

Once reaching the pericardium the short tail of the cercaria is lost in about ten days and they develop into unencysted metacercaria. Maturation of metacercaria may require as long as one year. Undeveloped testes and ovaries can be seen even in the metacercarial stage. Up to 90 percent or more of the susceptible snails may be naturally infected with metacercaria whereas only about 1.1 percent of all susceptible snails will harbor the sporocyst stage of the life cycle.

Mice and chipmunks become infected when eating snails infected with mature metacercaria. About 6 hours after ingestion the metacercaria reaches the cecal region, attaches to the cecal wall and begins to feed on blood. The metacercarial stage develops into a sexually mature adult in approximately 8 days post infection but eggs do not appear in the feces of the host until 19 to 21 days after infection. A minimum of 39 weeks is required for the completion of the life cycle, longer than any other member of the family Brachylaemidae.

RESULTS

An examination of the soft body parts of 415 snails for statistical data revealed that there was only one parasitic intermediate stage present. Of all the Anguispira kochi strontiana examined from Green Island, only the metacercaria of Postharmostomum helicis was found. The sporocyst stage of the life cycle was not found. Twenty-six snails were collected and examined in March, 1977 to determine if the metacercaria overwintered in the snails pericardium. They were infected with metacercaria.

The fecal examinations performed on the Peromyscus leucopus captured on South Bass Island revealed that the mouse passed nematode eggs but no digenetic trematode eggs were found. After the mouse was experimentally fed 5 A. kochi strontiana every other day for three weeks, a post mortem examination revealed that the mouse contained 42 adult P. helicis in the ceca and 2 adult capillarid nematodes. Five metacercaria were found in the stomach and intestine of the mouse. No other parasites were found.

The experimental infections using the metacercaria from A. kochi strontiana conducted with the P. leucopus captured in Columbus and Mus musculus acquired from the zoology department of The Ohio State University also proved successful. In groups I, a total of 6 adult Postharmostomum helicis were obtained from the cecal regions of the mice. In groups II, a total of 11 adult P. helicis adults were

collected and no P. helioides were obtained in the control groups.

Statistical tests were performed utilizing the data obtained from the heights, diameters, weights and number of infected A. kochi strontiana. Table I shows the means and standard deviations for heights, diameters, weights of the A. k. strontiana and the mean infection of the snails with P. helioides. The table also includes the incidence of infection in each zone and the density of snails per one meter square. The means measurements for height, diameter and weight are approximately the same for the four zones collected. The greatest variations can be seen in the mean infection, incidence of infection, and the snail density in each zone.

An analysis of variance test was performed to detect significant differences in incidence of infection between each zone at a confidence level of .05. The test revealed that all the zones except C and D, were significantly different from one another ($Pr > F = .0001$). The actual numbers of metacercaria found in zone A were 342, in zone B: 95, zone C: 748 and in zone D: 320.

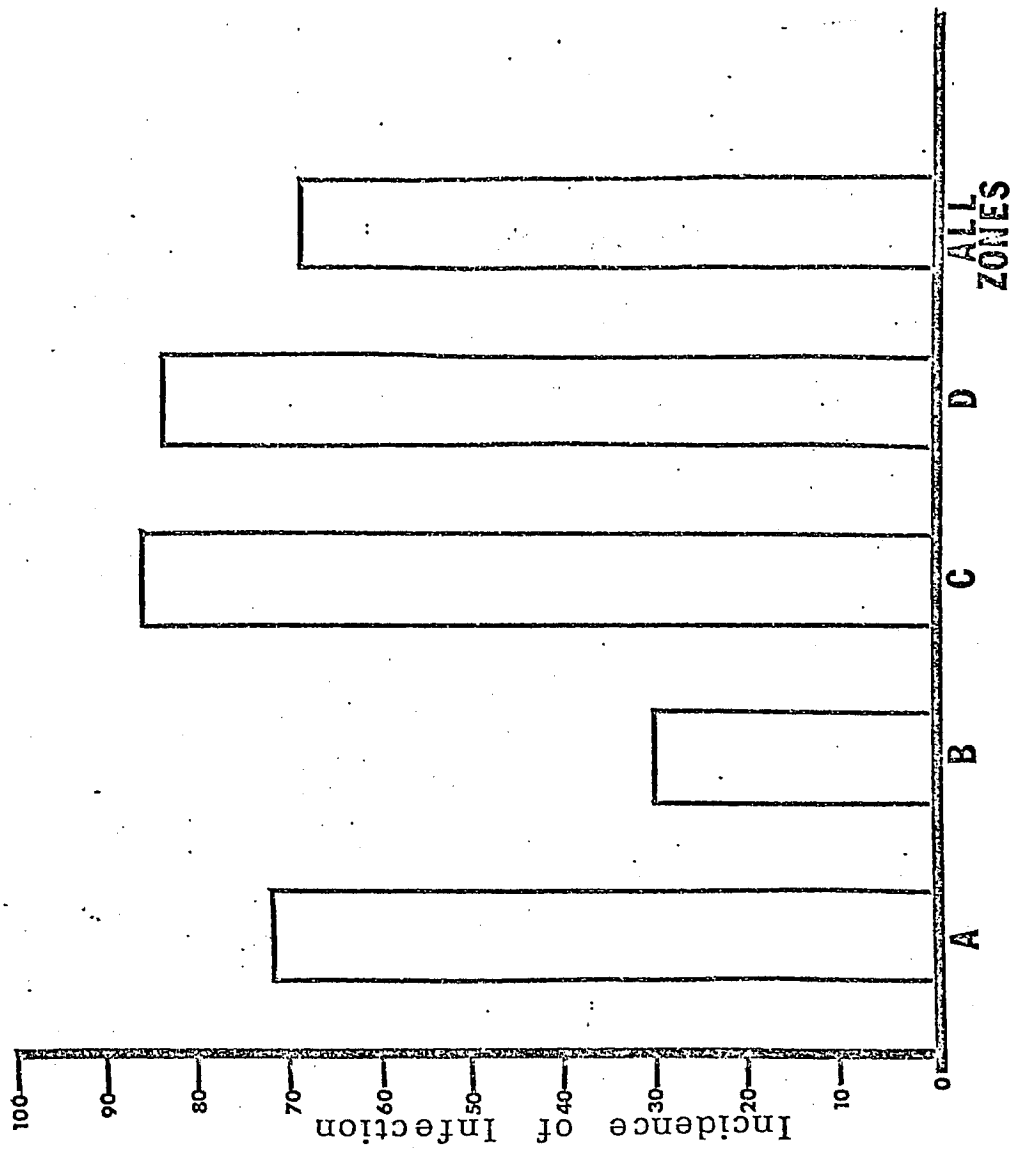
Text Figure 2 illustrates graphically the incidence of infection in each individual zone and in all the zones combined. The examination of the data for incidence of infection when combining all the zones revealed that there was a 68.2% infection present. Zone A had a 71% incidence, slightly over the mean incidence. Zone B had the lowest incidence of infection at 30% with zones C and D having approximately the same incidence of infection at 85.3% and 83.1% respectively.

An analysis of variance test was also performed to determine which parameter, height, diameter or weight had the most significant

Table 1. Means and standard deviations for height, diameter, weight and infection for snails with incidence of infection and density of snails in zones.

	<u>A</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>ALL</u>
Mean height	19.7 mm	19.0 mm	19.1 mm	20.0 mm	19.4 mm
Stand. dev.	2.62	2.35	1.76	1.56	2.15
Mean diameter	23.7	22.4	22.3	23.5	22.8
Stand. dev.	2.38	2.23	1.89	1.57	2.16
Mean weight	4.3 g	3.75 g	3.14 g	3.46 g	3.6 g
Stand. dev.	2.87	.98	1.81	1.75	1.97
Mean infection	3.42	.95	4.97	4.91	3.63
Stand. dev.	4.98	2.14	4.39	4.74	4.24
Incidence of infection	71%	30%	85.3%	83.1%	68.2%
Density 1 meter square	41 (h)* 12 (l)	14	49	45	34.1

* (h) = high density zones
(l) = low density zones



Text Figure 2. Percent Incidence of Infection in All Zones.

statistical fit with infection of the snail. At a .05 confidence level, height had the most significant statistical fit with infection for all zones combined ($\text{Pr}>F=.02$). When examining the zones individually one finds that in zone A, height was the only significantly different parameter ($\text{Pr}>F=.03$), in zone B none of the parameters were significantly greater than the others, in zone C height was once again significant ($\text{Pr}>F=.02$) and in zone D none of the parameters were significant.

An examination of all the snail correlation coefficients reveals that using a confidence level of .05, there is a significant positive correlation between height and diameter ($\text{Pr}>|r|=.0001$), height and weight ($\text{Pr}>|r|=.0001$) and diameter and weight ($\text{Pr}>|r|=.0001$). They also show a high degree of significance between height and diameter ($r=.86$), height and weight ($r=.68$) and diameter and weight ($r=.77$). The correlation coefficients for height, diameter and weight versus infection also at a confidence level of .05, reveals that over all zones there is a significant positive correlation between height and infection ($\text{Pr}>|r|=.0001$) and diameter and infection ($\text{Pr}>|r|=.0008$). However they are of a relatively low degree, height $r=.22$ and diameter $r=.16$. There was no significant correlation between weight and infection.

In zone A there was no significant correlation, positive or negative, between any of the parameters and infection. In zone B there was a significant positive correlation between height ($\text{Pr}>|r|=.02$), diameter ($\text{Pr}>|r|=.03$), and weight ($\text{Pr}>|r|=.02$) versus infection. The correlation between these parameters and infection are of a low degree, height versus infection ($r=.23$), diameter versus infection ($r=.21$), and weight versus infection ($r=.23$). In zone C there is a significant

positive correlation between all parameters and infection (height $\text{Pr}>|r|=.0001$, diameter $\text{Pr}>|r|=.0003$, weight $\text{Pr}>|r|=.0001$) but again at a low degree (height $r=.36$, diameter $r=.29$, weight $r=.32$). An examination of zone D reveals that the only significant correlation is that of weight versus infection ($\text{Pr}>|r|=.03$), once again at a low degree of correlation ($r=.28$).

Multiple factor regression equations of each zone are given below:

Multiple Factor Regression Equations by Zone

Zone A # of infections = $5.69 + .89(\text{ht.})^* - .88(\text{dia.}) + .26(\text{wt.})$

Zone B # of infections = $-1.81 + .09(\text{ht.}) + .01(\text{dia.}) + .33(\text{wt.})$

Zone C # of infections = $-8.23 + 1.17(\text{ht.})^* - .51(\text{dia.}) + .73(\text{wt.})$

Zone D # of infections = $10.27 - .64(\text{ht.}) - .15(\text{dia.}) + 3.16(\text{wt.})$

*denotes significant value

These regression equations were performed to estimate the number of infections when all the factors were working together. An examination of zone A reveals that height is the most significant factor in the equation ($\text{Pr}>|t|=.04$), the same is true of zone C ($\text{Pr}>|t|=.02$). The other zones B and D show no one factor significantly greater than any other factor.

In Table 2 are the frequencies of infection at specific heights in all zones combined. The greatest frequency occurs at 20 mm

which indicates that at this height the greatest probability of infection occurs.

Table 3 shows the frequency of infection at specific diameters in all zones. The greatest frequency for infection with metacercaria is at a diameter of 24 mm which indicates at this diameter the probability of infection is highest.

In Table 4 the frequencies of infection at specific weights in all zones are given. The highest percentage frequency of infection occurs at a weight of 3.5 g.

Tables 5 through 8 show the mean worm burdens, their standard deviations and frequency of infection at specific heights for zones A, B, C, and D. The mean worm burdens fluctuates from height to height and no general pattern can be demonstrated as to the overall increase or decrease of worm burdens as height increases. The frequency of infection demonstrates a different pattern. In each zone except zone A the frequency is highest at 20 mm. In zone A 21 mm has the highest frequency but the difference between the values is only slight.

In Tables 9 through 12 the mean worm burdens, standard deviations and frequencies of infection are given for specific diameters. Once again the mean worm burdens show no particular pattern and fluctuate as diameter increases. However the frequency of infection shows that in every zone except C the highest frequency is shown at a diameter of 24 mm. In zone C the highest frequency can be seen at 23 mm but the difference in frequencies between 23 mm and 24 mm is only slight.

Tables 13 through 16 show the mean worm burdens, standard deviations, and frequencies of infections of snails at certain weights. The mean worm burden from weight to weight once again fluctuates. The frequency of infection in zone A shows that in snails which weigh 4.5 g the frequency of infection is highest. In zone B the highest frequency of infection occurs at a weight of 4.0 g. Zones C and D show that at 3.5 g the highest frequency of infection occurs but in zone D the difference of frequency values between 3.5 g and 4.0 g is only slight.

Table 2. Percent frequencies for infection at certain heights in all zones.

<u>Height</u>	<u>Frequency</u>
13 mm	0.35%
16	0.35
17	5.3
18	12.7
19	20.5
20	29.3
21	20.9
22	7.8
23	2.5

Table 3. Percent frequency of infection at certain diameters in all zones.

<u>Diameter</u>	<u>Frequency</u>
16 mm	0.35%
18	0.35
19	0.35
20	4.6
21	5.3
22	19.1
23	21.9
24	31.1
25	13.8
26	2.5
27	0.35
28	0.35

Table 4. Percent frequency of infection at certain weights in all zones.

<u>Weight</u>	<u>Frequency</u>
1.5 g	0.35%
2.0	1.7
2.5	13.0
3.0	12.7
3.5	24.0
4.0	21.6
4.5	18.7
5.0	6.4
5.5	1.1
6.0	0.35

Table 5. Mean worm burdens, standard deviations and percent frequencies of infection for the height of snails in zone A.

<u>Height</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
11 mm	0.0	0.0	0.0%
13	0.0	0.0	0.0
16	3.50	4.95	1.4
17	2.75	3.4	1.4
18	2.90	3.25	9.7
19	3.53	4.22	19.4
20	2.77	3.12	23.6
21	4.04	7.33	25.0
22	3.10	1.91	12.5
23	6.50	10.11	4.2

Table 6. Mean worm burdens, standard deviations and percent frequencies of infection for the height of snails in zone B.

<u>Height</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
11 mm	0.00	0.00	0.0%
12	0.00	0.00	0.0
13	0.00	0.00	0.0
14	0.00	0.00	0.0
15	0.00	0.00	0.0
16	0.00	0.00	0.0
17	0.00	0.00	0.0
18	0.85	1.35	17.3
19	0.63	1.67	13.8
20	1.11	2.56	31.0
21	1.55	2.89	25.1
22	1.75	2.22	10.4
23	3.00	0.0	3.5

Table 7. Mean worm burdens, standard deviations and percent frequencies of infection for the height of snails in zone C.

<u>Height</u>	<u>Mean worm burden per individual</u>	<u>Worm burden Stand. dev.</u>	<u>Frequency</u>
13 mm	0.50	1.00	0.8%
16	0.00	0.00	0.0
17	2.39	3.22	8.6
18	4.05	4.99	11.7
19	5.43	5.09	27.3
20	6.10	4.19	30.5
21	6.05	3.87	15.6
22	5.50	3.39	4.7
23	21.0	0.00	0.8

Table 8. Mean worm burdens, standard deviations and percent frequencies of infection for the height of snails in zone D.

<u>Height</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
16 mm	0.00	0.00	0.0%
17	9.00	9.90	3.7
18	1.91	1.45	16.7
19	4.17	3.92	9.3
20	5.65	4.25	33.3
21	6.00	5.53	25.9
22	7.20	7.66	7.4
23	2.50	3.32	3.7

Table 9. Mean worm burdens, standard deviations and percent frequencies of infection for the diameter of snails in zone A.

<u>Diameter</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
15 mm	0.0	0.00	0.0%
18	0.0	0.00	0.0
19	0.0	0.00	0.0
20	0.0	0.00	0.0
21	3.7	5.51	2.8
22	3.7	4.52	8.3
23	4.3	7.87	20.8
24	3.9	4.93	37.5
25	2.5	3.14	20.8
26	2.6	2.41	5.6
27	2.0	2.83	1.4
28	8.0	0.00	1.4
30	0.0	0.00	0.0

Table 10. Mean worm burdens, standard deviations and percent frequencies of infection for the diameter of snails in zone B.

<u>Diameter</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
15 mm	0.00	0.00	0.0%
16	0.00	0.00	0.0
17	0.00	0.00	0.0
18	0.00	0.00	0.0
19	0.00	0.00	0.0
20	0.00	0.00	0.0
21	0.00	0.00	0.0
22	0.65	0.93	27.6
23	1.30	2.74	24.1
24	1.20	2.55	37.9
25	2.00	1.83	10.4

Table 11. Mean worm burdens, standard deviations and percent frequencies of infection for the diameter of snails in zone C.

<u>Diameter</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
15 mm	0.0	0.00	0.00%
16	1.0	1.41	0.78
18	0.0	0.00	0.00
19	4.0	6.93	0.78
20	3.2	4.5	8.60
21	3.5	3.04	9.40
22	4.8	5.17	21.90
23	6.2	4.36	25.80
24	5.8	4.55	24.20
25	7.7	5.83	7.00
26	4.0	0.00	1.60

Table 12. Mean worm burdens, standard deviations and percent frequencies of infection for the diameter of snails in zone D.

<u>Diameter</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
18 mm	0.00	0.00	0.00%
19	0.00	0.00	0.00
20	4.25	7.85	3.70
21	0.67	1.16	1.90
22	3.42	2.02	22.20
23	4.39	3.73	12.96
24	5.10	4.01	35.20
25	7.90	6.4	22.20
26	3.00	6.0	1.90

Table 13. Mean worm burdens, standard deviations and percent frequencies of infection for the weight of snails in zone A.

<u>Weight</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
1.0 g	0.0	0.00	0.0%
1.5	0.0	0.00	0.0
2.0	0.0	0.00	0.0
2.5	0.0	0.00	0.0
3.0	1.9	2.67	5.6
3.5	3.6	3.48	11.1
4.0	4.7	7.79	23.6
4.5	2.9	3.05	37.5
5.0	4.3	6.46	18.1
5.5	2.5	3.21	3.27
8.5	0.0	0.00	0.0

Table 14. Mean worm burdens, standard deviations and percent frequencies of infection for the weight of snails in zone B.

<u>Weight</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
1.0 g	0.0	0.00	0.00%
1.5	0.0	0.00	0.00
2.0	0.0	0.00	0.00
2.5	0.0	0.00	0.00
3.0	0.0	0.00	0.00
3.5	1.35	3.00	17.24
4.0	0.8	1.42	41.38
4.5	1.6	2.87	34.48
5.0	1.0	0.00	3.45
6.0	3.0	0.00	3.45

Table 15. Mean worm burdens, standard deviations and percent frequencies of infection for the weight of snails in zone C.

<u>Weight</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
1.0	0.00	0.00	0.00%
1.5	1.00	1.40	0.78
2.0	1.59	2.64	3.12
2.5	3.68	4.40	21.09
3.0	6.26	4.45	18.75
3.5	5.81	4.90	27.34
4.0	6.61	3.40	14.06
4.5	5.57	4.22	10.15
5.0	11.00	14.14	1.56

Table 16. Mean worm burdens, standard deviations and frequencies of infection for the weight of snails in zone D.

<u>Weight</u>	<u>Mean worm burden per individual</u>	<u>Worm burden stand. dev.</u>	<u>Frequency</u>
1.0 g	0.00	0.00	0.00%
1.5	0.00	0.00	0.00
2.0	0.67	1.16	1.85
2.5	3.77	4.42	18.52
3.0	3.43	2.30	14.82
3.5	5.81	4.10	29.63
4.0	7.43	6.02	27.78
4.5	6.30	6.03	3.70
5.0	3.25	4.72	3.70

DISCUSSION

The pulmonate snail Anguispira kochi strontiana (Figure I) is by far the most abundant land snail on Green Island. This subspecies of Anguispira kochi is found only among the isles of Lake Erie. In an attempt to find other living species of snails, Green Island was thoroughly searched. An extensive search revealed thousands of living A. kochi strontiana, hundreds of Limax maximus, and only a single specimen of the shell-bearing land snail, Haplotrema concavum. No parasitic stages were found in L. maximus or H. concavum. Shells of various species of snails could be found in piles on Green Island but none were alive.

When examining the old shells on the island, I found that at some time in the history of the island Anguispira alternata was present on the island but no living representatives exist at this time. It could be postulated that because A. k. strontiana was better selectively adapted to the conditions and the terrain on Green Island and that it could have crowded out A. alternata and other shell-bearing land snails.

Of the 415 snails examined for statistical data a total of 1505 Postharmostomum helicis metacercariae were recovered from the pericardia. There was a total of 132 uninfected snails with the majority of the uninfected ones coming from zone B. Twenty-six snails from Green Island examined in March of 1977 also contained metacercariae.

This indicates that the metacercariae are overwintering with the snail. Approximately 700 snails were actually examined and either contained P. heliciis metacercariae in the pericardium or were uninfected. No sporocyst stage of P. heliciis was found.

Although the sporocyst stage was not observed, the numerous infections of the second intermediate hosts would indicate that snails harboring the sporocysts are present on the island. Ulmer (1951) indicates that the sporocyst stage of P. heliciis only occurs in approximately 1.1% of the population and indicates that size of the snail is of little significance. He however was describing the sporocyst infection among A. alternata. It is my belief that even if very few numbers of A. alternata were present on Green Island, it could not account for the great infection rate of metacercariae present in the second intermediate hosts on the island.

The effect of the parasites presence in the pericardial region of the snail was not determined in this study, although in some cases snails which harbored metacercariae had the same heights, diameters, and weights as non-infected snails. In several snails dead metacercariae were found, with some snails harboring living metacercariae as well as dead ones. The dead metacercariae were apparent because of their opaque color and their still nature. The dead specimens could have been older metacercariae that expired due to an extended stay within the pericardium or they may have been cercariae that did not develop.

A maximum of 36 metacercariae were recovered from a single snail and the pericardium seemed to be as healthy as the pericardium of uninfected snails. No apparent damage could be seen by general observation. In this extreme case however, the region did appear to be swollen or

enlarged due to the presentance of such large numbers of metacercaria. I do not feel that the enlargement was due to tissue reaction by the snail to the presence of the parasite. The pericardial regions of snails with relatively low infections (1 to 4 worms) did not show any abnormalities by general observation.

Kennedy (1975) points out that because in this type of life cycle where the second intermediate host must be ingested, it would be of little advantage for the parasite to damage severely or kill its host before it can be ingested. In this way the parasite may exist in its host for substantial periods of time.

To confirm the identification of the distome trematode found, feeding experiments were conducted. The experimental infections were successful. All of the mice except one that were fed metacercaria from A. k. strontiana harbored adult Postharmostomum helicis upon dissection of the digestive system. Infected animals could be detected almost immediately upon examination of the ceca. The walls of the ceca appeared to have ulceration, which were apparently due to the tenacious grip of the suckers and the feeding process of the parasites.

Adult P. helicis that were taken from the cecum of the experimentally infected mice were examined alive and after fixing stained specimens on slides. Figure 3 shows a drawing of the adult trematode of this species. The species found has been confirmed as Postharmostomum helicis for the following observations:

- 1) The length and width measurements of the 20 adult trematodes body, oral sucker, acetabulum, and eggs vary only slightly with the measurements published by Robinson (1949b) and Ulmer (1951).

Mean body length: 2.9 mm
Mean body width: 1.17 mm
Oral sucker length and width: 0.46 mm x 0.49 mm
Acetabulum length and width: 0.40 mm x 0.42 mm
Eggs length and width: 0.03 mm x 0.017 mm

Adults were determined by maturation of genitalia and eggs in the uterus.

2) The arrangement of the genitalia, the ovary and testes, are in a triangular pattern with the genital pore located in the medial plane of the body just above the ovary and anterior testes.

3) The vitellaria, which are sparse and follicular, extends from the pharynx to just above the ovary and testes. The adults also possess a prominent vitelline reservoir which is located in the posterior portion of the body between the ovary and anterior testes.

4) A smooth cuticle can be observed in the metacercaria (Figure 3) and adult stages.

5) The convolutions, which are usually 6 in number, of the ceca wind from the muscular pharynx to the posterior end of the body. This distinct characteristic can be observed in the metacercaria and the adult.

6) The metacercaria measurements described by Ulmer are also similar to the metacercaria found in A. k. strontiana.

Mean body length: 0.93 mm

In order to compare infections in different areas of Green Island, snails were collected and examined from different geographical zones of the island. The zones chosen represented areas which were found on both ends of the island and in the middle of the island.

The selection of these particular zones were made because they tend to give a better idea of what is occurring on most of the island and in these regions snails were abundant.

When examining the vegetation of each zone on the island, one finds both striking similarities and differences. Zone A was located on the middle of the island but the overall length of this zone covered almost half of the island. Collections of snails were made not more than 10 meters off the concrete walkway (Text Figure 1). The vegetation in this zone was mainly composed of sugar maple, hackberry, ash and cottonwood trees with many old rotten windfalls distributed throughout the zone. There was also moderate amounts of tall grasses and shrubs. A heavy population of snails seemed to be distributed throughout the zone. An average of 41 snails per square meter were found in areas of high density and 12 per square meter in areas of low density.

Zone B was on the extreme west end of the island where the vegetation was more dense than in the other zones and there were fewer growing trees or rotting logs. In this zone there was more ground cover and more species of herbaceous plants than in the other zones. In zone B the population of snails was by far the least dense. An average of 14 snails per square meter was recorded in this zone.

The third zone, zone C, was located approximately in the center of the island about 30 meters from walkway. The vegetation found in this region was much the same as in zone A, with a number of downed trees in the immediate area. This zone had an area of 5 to 10 square meters in an area where the population had the highest density. An average of 49 snails was recorded in an area one meter square.

Zone D was found on the extreme eastern shore of the island. The vegetation was much the same as found in zones A and C. Snails in this zone seemed to have a discontinuous distribution. There were densely populated regions and areas between these regions where no snails were found. Zone D snails were collected in a highly populated region with an area of about 5 to 10 square meters. In these regions an average of 45 snails per square meter was recorded giving this zone the second highest density of all the zones.

Text Figure 2 correlates incidence of infection in zones A, B, C, D, and the mean incidence for all zones combined. The highest incidence of infection can be seen in zones C and D, with zone C slightly higher. These zones were not significantly different. It is postulated that because the snails from zones C and D were collected from highly populated regions that their contact with each other within the zone was greatest, thus increasing the chances of coming in contact with the first intermediate host which contained the sporocyst.

Because the vegetation and terrain of zones C and D were nearly the same this could also account for their similarities in incidence of infection. For example, the large number of fallen trees found in each zone provide excellent nesting sites for Peromyscus sp. and the sugar maple leaves along with other vegetation provide excellent food for the snails. These zones also contained rock outcrops for cover and housing.

Zone B shows the lowest incidence of infection in Text Figure 2. This zone was statistically different from all other zones. The fact that the snails were not as dense or as highly populated would suggest that the contact among individuals would not be as great as those in

zones C or D. This would lessen its chances of also crossing the slime trail of infected first intermediate host. There were few fallen logs, not as many growing large trees and a greater abundance of thickened ground cover. These facts would decrease the nesting habitate of P. leucopus and decrease the food available for A. kochi strontiana.

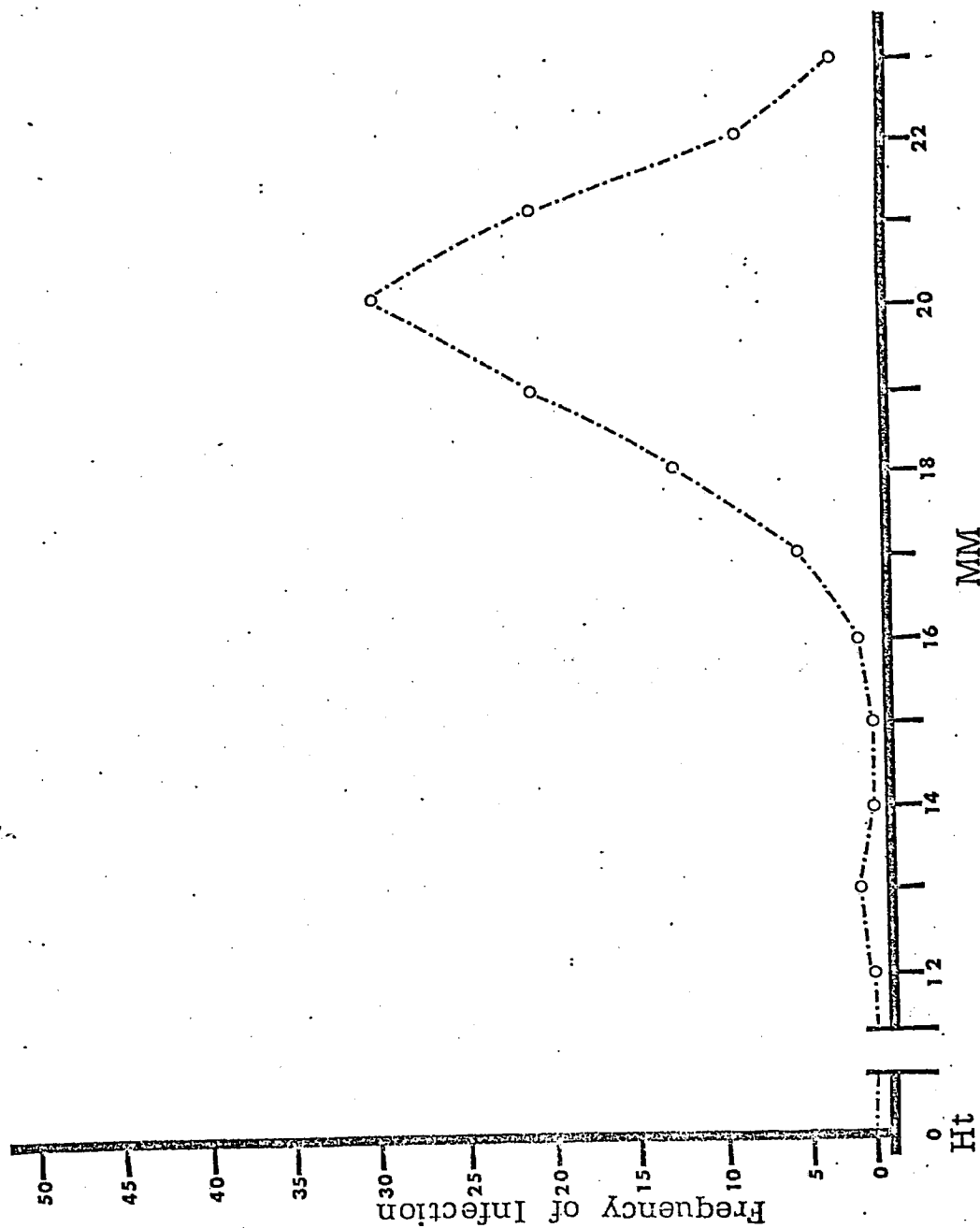
The incidence of infection in zone A was found significantly different from zones B, C, and D. Its incidence is nearly the same as that of all the zones combined. This zone's vegetation seemed to provide a good habitat for both intermediate and definitive host with its fallen rotten logs, low grasses, rock outcrops, and tall trees. The snails in this zone were collected from highly populated areas which was continuous with sparsely populated areas. This combination, areas of dense population of snails combined with areas of less dense population of snails, could account for the lessor incidences of metacercariae in zone A as compared with zone C and D. This also may explain why zone A has an incidence of infection similar to the mean value of all zones.

The frequency of infection correlated with heights of infected A. k. strontiana in all zones combined is contained in Text Figure 3. It illustrates a negative binomial frequency distribution with the largest frequency occurring at a height of 20 mm. The graph also shows that snails with heights less than 17 mm were rarely infected with metacercaria, whereas snails larger than 22 mm were rarely infected also. This would indicate that in any zone a snail with a height from 18 to 22 mm has the greatest chance of being infected with metacercaria.

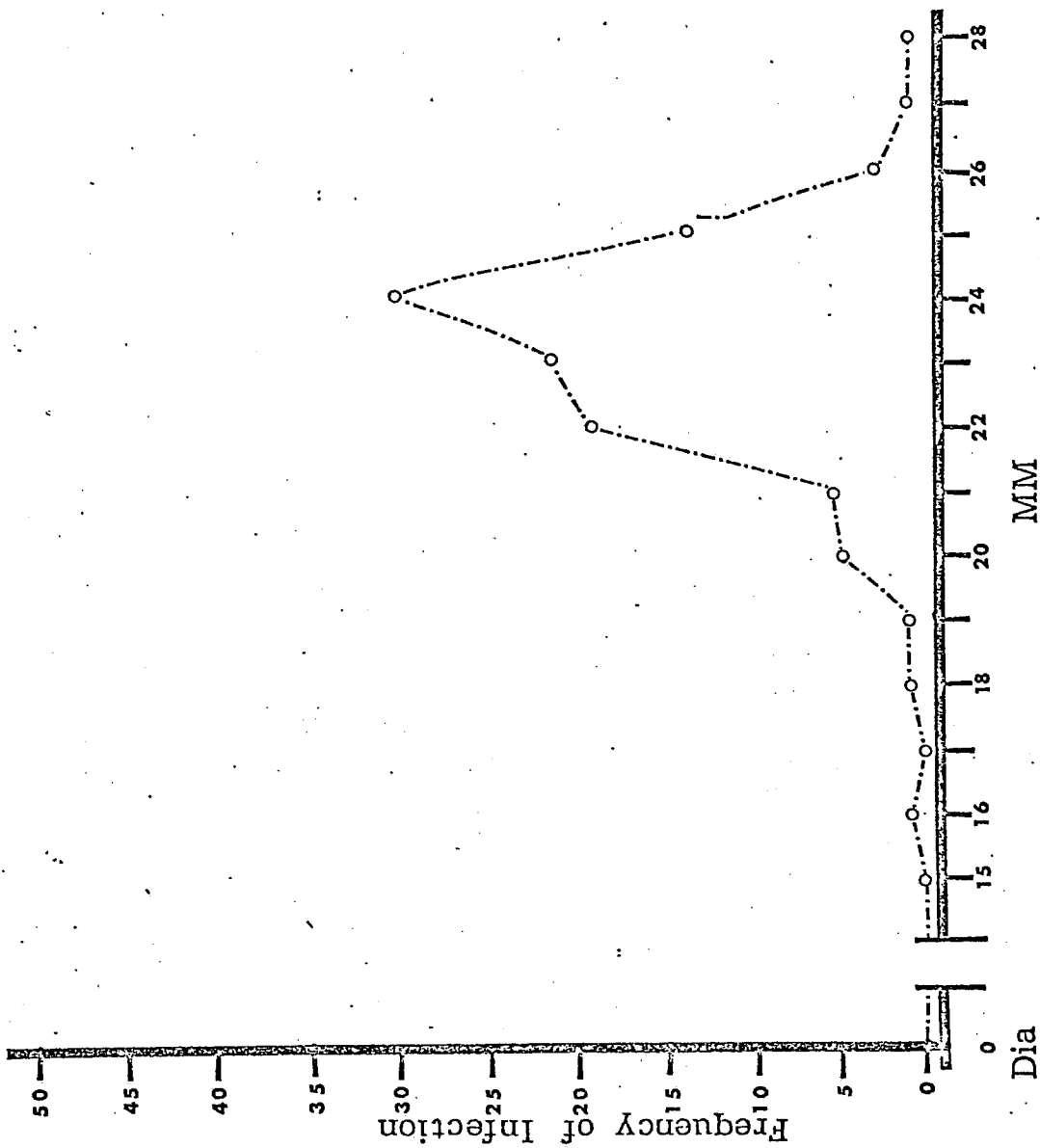
When examining the frequency of infection correlated with the diameter of the snails in all zones combined (Text Figure 4), one finds it too has a negative binomial frequency distribution curve. The peak of the curve occurs at a diameter of 24 mm. The graph shows that snails with a diameter of less than 21 mm and greater than 26 mm were rarely infected. Snails with diameters of 21 to 25 mm show the greatest probability of infection with P. helioides metacercaria.

Text Figure 5 correlates frequency of infection with the weight of the snail in all zones. The weights were rounded to the nearest 0.5 g. The frequency once again has a negative binomial distribution with the peak of the curve occurring at 3.5 g. In this graph, snails that weigh less than 2.5 g and more than 5.0 g have the lowest frequency of infection. Snails that weigh between 2.5 and 5.0 g have the greatest probability of infection. The frequency of infection curves show that a snail found in any zone with a height ranging from 18 to 22 mm, diameter ranging from 21 to 25 mm, and a weight from 2.5 to 5.0 g would have the greatest probability of being infected.

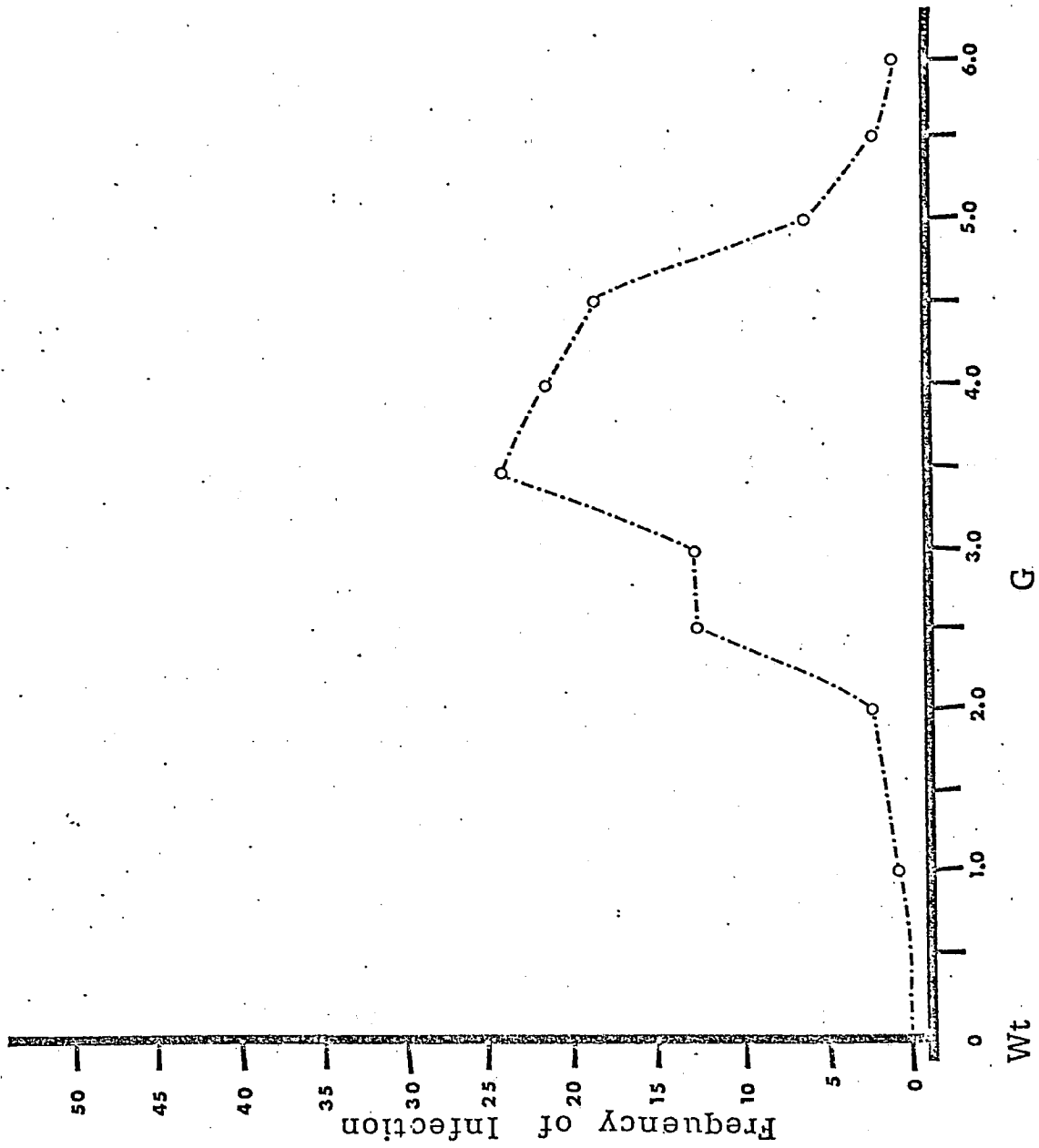
The graphs which illustrate frequency of infection for height, diameter, and weight show that snails which are smaller or larger than those in the infection range have the lowest percentage frequency of infection. It is not known why this phenomenon occurs but it is postulated that the smaller snails are less suitable for infection or do not behave the same as older snails. The older snails (snails larger than those in the greatest infection ranges) may build up an immunity to infection by metacercaria as they get older.



Text Figure 3. Percent Frequency of Infection Versus Height in All Zones Combined.



Text Figure 4. Percentage Frequency of Infection Versus Diameter in All Zones Combined.

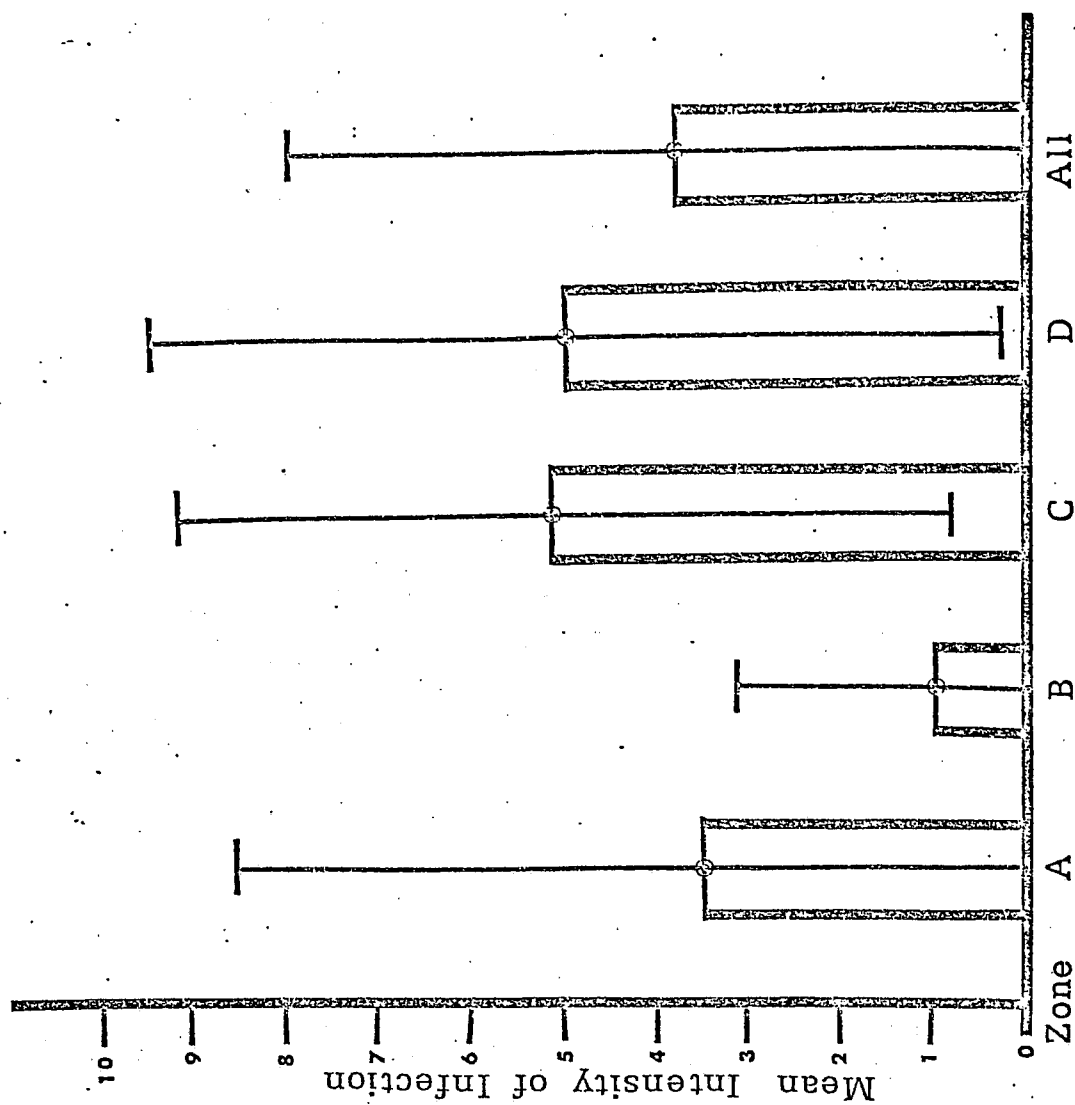


Text Figure 5. Percent Frequency of Infection Versus Weight in All Zones Combined.

A graphic illustration of the mean intensity of infection of the zones is shown in Text Figure 6. Their standard deviations are also included. The highest intensity occurs in zones C and D and they are once again approximately the same. The lowest intensity is seen in zone B, with zone A once again resembling that of all zones combined. When examining the intensity at specific heights, diameters, and weights one finds that the distribution fluctuates as measurements increase (Tables 5 through 16). Another observation is that the peak in frequency of infection and intensity of infection do not necessarily coincide with in zones. I feel that the intensity may be affected by the density of the snail population in a specific zone. It is interesting also to note that the zone with the highest incidences of infection also have the highest mean intensity of infection (Table 1).

Throughout all the zones height seemed to have the most significant statistical fit involving infection of the snails. It is interesting to find that the two zones where height was of less significance than the other parameters, zones B and D, occur on opposite ends of the island.

When examining the correlation coefficients for all zones combined, they show that there is a significant positive correlation suggesting that as the height of the snail increases so does the diameter and weight. The same is true with diameter and weight, as diameter increases so does weight. This would indicate that as the height of the spire of the snail increases, so does the diameter and weight of the snail. It is generally recognized by zoologists that the height of spires on mollusk shells represent growth and age of the animal.



Text Figure 6. Mean Intensity of Infection Versus Zone.

Vertical lines represent standard deviations of means.

In view of the correlations it should be safe to say that as the spire of the snail increases with time so does the diameter and weight of the snail, thus a correlation with age of snails is also achieved.

In zone A, the growth correlations (correlation between height, diameter, and weight) were significant at high degrees also, but this zone showed no significant statistical correlation between any of these lone parameters and infection. This uncorrelated effect is unexplained at the present time.

Zone B shows that the correlations show a positive correlation of a high degree. The relationship between height and infection, diameter and infection and weight and infection are positive correlations at low degrees and almost at the same levels of significance. This is an indication that snail within certain ranges of height, diameter, and weight are most likely to be infected.

Zone C shows that the correlations are positive and significant as in the two previous zones and show a significant relation of low degree with height and infection, diameter infection, and weight versus infection. In this zone because of the similarities in the r values each factor is contributing a significantly close statistical fit, although height does show the greatest degree of correlation.

Zone D shows positive significant correlation of growth parameters to a high degree but the only parameter that appears to significantly correlate statistically with infection is weight. This is different from any other zone.

Combined data of all zones combined show the significant positive correlation of a high degree when growth parameters are involved.

The zones do not however share the correlations with lone parameters versus infection. This would indicate that because the zones are different in location, vegetation and general terrain each zone may have other factors which affect the infection of the second intermediate host.

A multiple factor regression equation for each zone was performed to estimate the number of parasites in a snail with a specific height, diameter, and weight in a specific zone. These regression equations serve as models and help to further illustrate the similarities and differences in the zones. Height is the only significant factor and it is only significant in zones A and C, the zones located on the middle of the island. Zones B and D, located at opposite ends of the island along the shore, had no one factor with a significant statistical fit. When a factor is not significant in the equation this indicates that its value could be zero or that it has little effect on the equation estimation.

Zones A and C yield a better fit for height of the snail with infection rather than any other factor, whereas zones B and D are using the three parameters at approximately the same level of statistical fit with infection.

The success or failure of the completion of the life cycle of Postharmostomum helicis evolves around a combination of many complex factors in its environment. The life cycle seems to be based on the general contact of intermediate hosts and definitive host, and the feeding habits of the hosts. According to the R-square factor, 17% of the infection of the second intermediate host is accounted for by the

parameters height, diameter, and weight in all zones. This is a moderate percentage level but it suggests that these parameters do give an illustration of infection rates in the second intermediate host.

CONCLUSIONS

1. Anguispira kochi strontiana is by far the most abundant shell-bearing land snail on Green Island.
2. Postharmostomum helicis metacercaria were found in the pericardium of A. k. strontiana and is the first report of any parasite in this subspecies.
3. Postharmostomum helicis was the only parasite found in the representative A. k. strontiana examined from Green Island.
4. Postharmostomum helicis metacercaria do overwinter in the A. k. strontiana on Green Island.
5. Metacercaria from A. k. strontiana fed to Peromyscus leucopus developed into adult Postharmostomum helicis.
6. No parasitic stages were found in Limax maximus on Green Island.
7. Statistical correlations coefficients of height, diameter, and weight of A. k. strontiana are highly significant.
8. The incidence of infection of snails harboring Postharmostomum helicis metacercaria in all zones combined was 68.2%.
9. A. k. strontiana collected from regions with seemingly high densities have the highest incidences of infection.
10. The mean intensities of infection for specific heights, diameter, and weights in A. k. strontiana do not correlate with the percentage frequency of infection for specific heights, diameters, and

weights, although the highest mean intensity can be seen in those zones which have the highest incidence of infection.

11. The frequency of infection curves for height, diameter, and weight show negative binomial distributions for the snail population examined.
12. Snail with a height of 18 to 22 mm, a diameter of 21 to 25 mm and a weight of 2.5 to 5.0 g show the greatest frequency of infection for all zones combined.
13. Analysis of variance tests reveal that the height of the snail has the most significant statistical fit with infection of second intermediate host.
14. Multiple factor regression equations can provide valid estimates of metacercariae in a snail from a specific zone with a specific height, diameter, and weight.
15. R-square factors reveal that only 17% of the infection of the second intermediate host is explained by the parameters height, diameter, and weight.

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EXPLANATION OF FIGURE I

Anguispira kochi strontiana from Green Island.

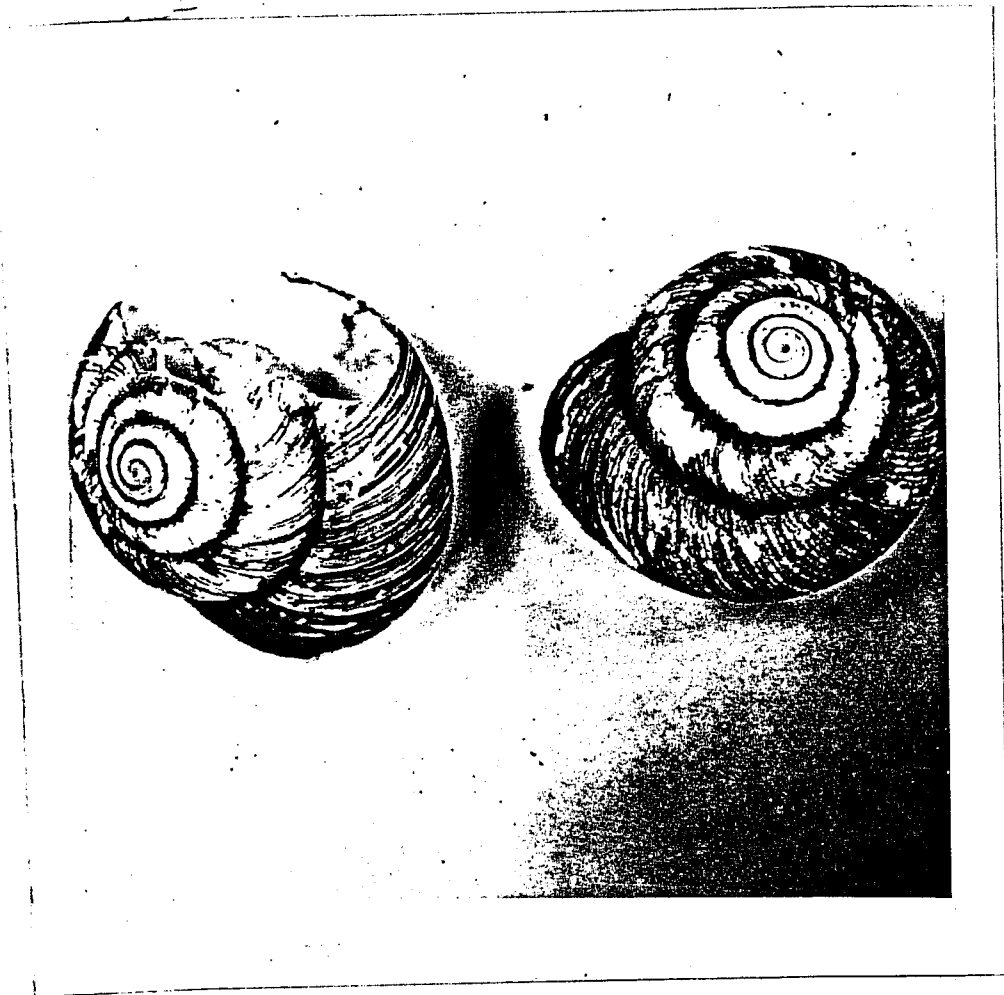


FIGURE I

EXPLANATION OF FIGURE II

Adult Postharmostomum helicis from the ceca of Peromycus leucopus.

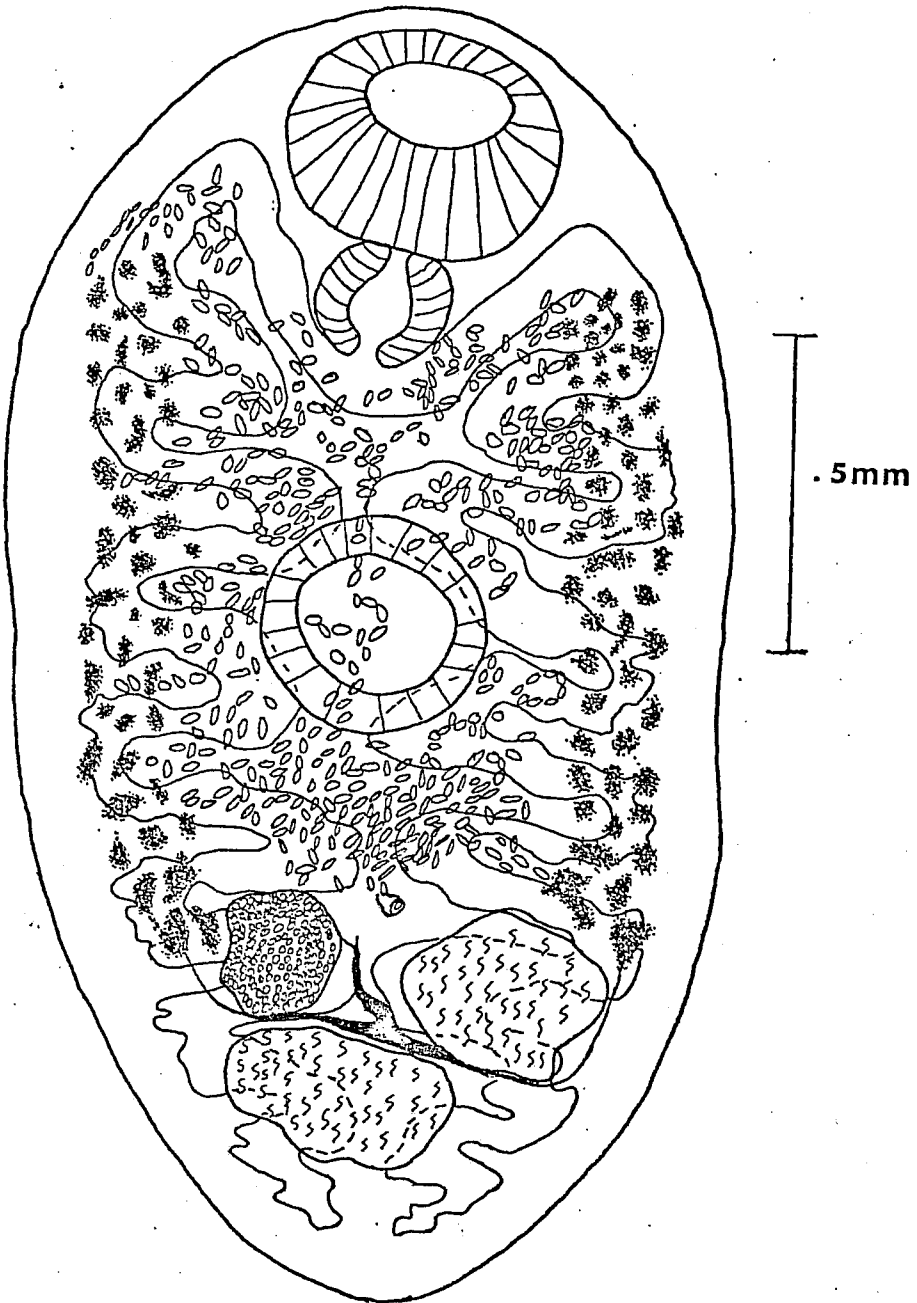


FIGURE II

EXPLANATION OF FIGURE III

Mature metacercaria of Postharmostomum helcis from the pericardium of Anguispira kochi strontiana.

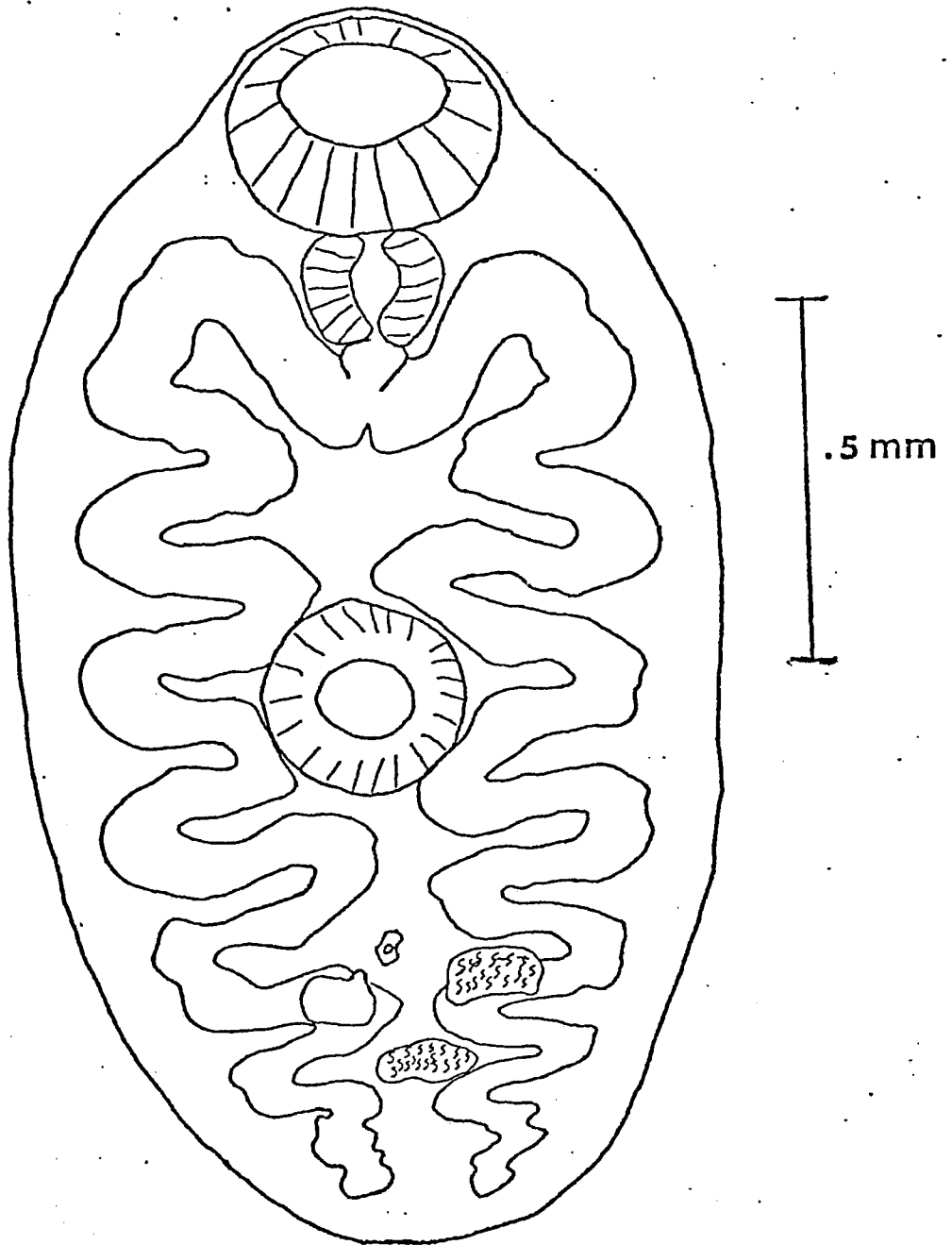


FIGURE III